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The Mismatch Repair System Reduces Meiotic Homeologous Recombination and Stimulates Recombination-Dependent Chromosome Loss

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Efficient genetic recombination requires near-perfect homology between participating molecules. Sequence divergence reduces the frequency of recombination, a process that is dependent on the activity of the mismatch repair system. The effects of chromosomal divergence in diploids of *Saccharomyces cerevisiae* in which one copy of chromosome III is derived from a closely related species, *Saccharomyces paradoxus*, have been examined. Meiotic recombination between the diverged chromosomes is decreased by 25-fold. Spore viability is reduced with an observable increase in the number of tetrads with only two or three viable spores. Asci with only two viable spores are disomic for chromosome III, consistent with meiosis I nondisjunction of the homeologs. Asci with three viable spores are highly enriched for recombinants relative to tetrads with four viable spores. In 96% of the class with three viable spores, only one spore possesses a recombinant chromosome III, suggesting that the recombination process itself contributes to meiotic death. This phenomenon is dependent on the activities of the mismatch repair genes *PMS1* and *MSH2*. A model of mismatch-stimulated chromosome loss is proposed to account for this observation. As expected, crossing over is increased in *pms1* and *msh2* mutants. Furthermore, genetic exchange in *pms1 msh2* double mutants is affected to a greater extent than in either mutant alone, suggesting that the two proteins act independently to inhibit homeologous recombination. All mismatch repair-deficient strains exhibited reductions in the rate of chromosome III nondisjunction.

Homologous recombination occurs most frequently between identical DNA sequences. Studies with a variety of organisms have demonstrated that sequence divergence reduces the frequency of recombination (7, 16, 19, 33, 53, 54, 60, 73, 75). Recombination between two similar but nonidentical sequences (termed homeologous sequences) will form a heteroduplex intermediate containing mispaired DNA. Mismatches formed during recombination are corrected by the mismatch repair system, which provides the molecular basis of gene conversion (4). In *Escherichia coli*, the long-patch mismatch repair system is the main pathway for correcting errors that arise during DNA replication (reviewed in reference 58). The suppression of recombination between homeologous sequences is strongly dependent on elements of this system (14, 16, 33, 59, 60, 69). The MutS, MutL, MutH, and UvrD proteins are the central components of the *E. coli* mismatch repair system. MutS binds to mismatches in duplex DNA. MutL couples MutS to the endonuclease MutH, which nicks the nascent DNA strand, and the helicase UvrD is thought to displace the targeted strand (reviewed in reference 19). Numerous homologs of the *E. coli mutS* and *mutL* genes have been identified in eukaryotes. Three *mutL* (*MLH1*, *MLH2*, and *PMS1*) (40, 56) and six *mutS* (*MSH1* to *MSH6*) (30, 46, 52, 61, 62, 66) homologs have been identified in the yeast *Saccharomyces cerevisiae*. Studies of these genes indicate that only a subset participates in nuclear mismatch repair. *pms1*, *mlh1*, *msh2*, *msh3*, and *msh6* mutants all display a mutator phenotype consistent with a role in the repair of errors arising during DNA synthesis (46, 56, 61, 78). *pms1*, *mlh1*, and *msh2* mutants display high frequencies of postmeiotic segregation (PMS) of genetic markers (56, 61, 78), indicative of failure to correct mismatches in heteroduplex DNA formed during recombination. *msh3* and *msh6* mutants also display a PMS phenotype, although to a lesser degree (6, 52). *MSH1* has an exclusively mitochondrial function (62), while *MSH4* and *MSH5* are believed to be involved in the maturation of meiotic interhomolog crossovers but have no apparent role in mismatch repair (30, 66).

In eukaryotes, recombination is observed at its highest frequencies during meiosis. At least one crossover per chromosome is generally required to ensure segregation; failure to cross over often results in undirected segregation and increased frequencies of nondisjunction. Several studies have demonstrated that decreased homology between chromosomes reduces meiotic recombination (33, 53, 54). A diploid of *S. cerevisiae* with one chromosome III derived from *Saccharomyces carlsbergensis* shows a dramatic contraction of map distance on the left arm of the chromosome (54). The exchange that occurs is limited to the *MAT-THR4* interval on the right arm of chromosome III. Physical and genetic analysis of genes on this (31) and other (9, 10, 25, 36, 53) chromosomes reveals that *S. carlsbergensis* possesses two corresponding chromosome types, one which recombines at wild-type frequencies with an *S. cerevisiae* tester strain, and another which exhibits virtually no exchange. The chromosome III used in the above study is thought to be a mosaic of *S. cerevisiae* and *S. monacensis* sequences, with regions of high homology where recombination is normal and regions of low homology with no crossing over. These experiments, therefore, do not adequately investigate the recombination and segregation of a completely homeologous chromosome pair.

The effects of chromosomal divergence on meiosis have also been examined by using an interspecific hybrid diploid of *S. cerevisiae* and *S. paradoxus* (also called *Saccharomyces douglasii* [28, 33]). The two species appear to be closely related by several criteria. *S. paradoxus* has a karyotype similar to that of

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TABLE 1. Strains used in this study

Strain	Genotype[a]
Y55	*S. cerevisiae HO* wild type
N17	*S. paradoxus HO* wild type
Haploids	
Y55 2291	*MATa his4-r leu2-r thr4-a ade1-1 ura3-n met13-4 lys2-d can1-1*
Y55 2395	*MATα kar1-Δ13 ade1-1 his4-r leu2-r thr4-a ura3-n met13-4 cyh2-1*
SCt2	*MATa HML::ADE1 his4-r leu2-r thr4-a ade1-1 ura3-n met13-4 lys2-d can1-1*[b]
SCt14	*MATα HIS4 LEU2 THR4 kar1-Δ13 ade1-1 ura3-n met13-4 cyh2-1*[c]
Diploids	
SCD 22	*MATa HML::ADE1 his4-r leu2-r thr4-a*[b] *KAR1 ade1-1 can1-1 ura3-n met13-4 CYH2 lys2-d*
	MATα HML HIS4 LEU2 THR4[c] *kar1-Δ13 ade1-1 CAN1 ura3-n met13-4 cyh2-1 LYS2*
SCD 28	*MATa HML::ADE1 his4-r leu2-r thr4-a*[b] *KAR1 ade1-1 trp1-b can1-1 ura3-n met13-4 CYH2 lys2-d*
	MATα HML HIS4 LEU2 THR4[c] *kar1-Δ13 ade1-1 TRP1 CAN1 ura3-n met13-4 cyh2-1 LYS2*
SCD 23	*MATa HML::ADE1 his4-r leu2-r thr4-a*[b] *KAR1 ade1-1 can1-1 ura3-n pms1Δ::URA3 met13-4 CYH2 lys2-d*
	MATα HML HIS4 LEU2 THR4[c] *kar1-Δ13 ade1-1 CAN1 ura3-n pms1Δ::URA3 met13-4 cyh2-1 LYS2*
SCD 29	*MATa HML::ADE1 his4-r leu2-r thr4-a*[b] *KAR1 ade1-1 trp1-b can1-1 ura3-n pms1Δ::URA3 met13-4 CYH2 lys2-d*
	MATα HML HIS4 LEU2 THR4[c] *kar1-Δ13 ade1-1 TRP1 CAN1 ura3-n pms1Δ::URA3 met13-4 cyh2-1 LYS2*
SCD 30	*MATa HML::ADE1 his4-r leu2-r thr4-a*[b] *KAR1 ade1-1 can1-1 ura3-n msh2::URA3 met13-4 CYH2 lys2-d*
	MATα HML HIS4 LEU2 THR4[c] *kar1-Δ13 ade1-1 CAN1 ura3-n msh2::URA3 met13-4 cyh2-1 LYS2*
SCD 37	*MATa HML::ADE1 his4-r leu2-r thr4-a*[b] *KAR1 ade1-1 can1-1 ura3-n trp1-b msh2::URA3 CYH2*
	MATα HML HIS4 LEU2 THR4[c] *kar1-Δ13 ade1-1 CAN1 ura3-n TRP1 msh2::URA3 cyh2-1*
SCD 40	*MATa HML::ADE1 his4-r leu2-r thr4-a*[b] *KAR1 ade1-1 can1-1 ura3-n pms1Δ msh2::URA3 CYH2 lys2-d*
	MATα HML HIS4 LEU2 THR4[c] *kar1-Δ13 ade1-1 CAN1 ura3-n pms1Δ msh2::URA3 cyh2-1 LYS2*
SCD 39	*MATa HIS4 LEU2 THR4 kar1-Δ13*[c] *ade1-1 CAN1 ura3-n met13-4 cyh2-1 LYS2*
	MATα HIS4 LEU2 THR4 kar1-Δ13[c] *ade1-1 CAN1 ura3-n met13-4 cyh2-1 LYS2*
Y55 control diploids	
SCD 24	*MATa HML::ADE1 his4-r leu2-r thr4-a ade1-1 ura3-n met13-4 lys2-d*
	MATα HML HIS4 LEU2 THR4 ade1-1 ura3-n MET13 LYS2
SCD 25	*MATa HML::ADE1 his4-r leu2-r thr4-a ade1-1 ura3-n pms1Δ::URA3 met13-4 lys2-d*
	MATα HML HIS4 LEU2 THR4 ade1-1 ura3-n pms1Δ MET13 lys2-d
SCD 35	*MATa HML::ADE1 his4-r leu2-r thr4-a ade1-1 trp1-b ura3-n lys2-d*
	MATα HML HIS4 LEU2 THR4 ade1-1 TRP1 ura3-n LYS2
SCD 38	*MATa HML::ADE1 his4-r leu2-r thr4-a ade1-1 trp1-b ura3-n msh2::URA3 met13-4 CYH2*
	MATα HML HIS4 LEU2 THR4 ade1-1 TRP1 ura3-n msh2::URA3 MET13 cyh2-1

[a] All diploids were created from SCt2 and SCt14 or mismatch repair mutant derivatives of SCt2 and SCt14.
[b] Chromosome III derived from *S. cerevisiae*.
[c] Chromosome III derived from *S. paradoxus*.

S. cerevisiae, although minor chromosomal size differences can be detected by contour-clamped homogeneous electric field (CHEF) gel analysis (51). The 15 genes examined to date by hybridization analysis are located on the same chromosomes in both species (33, 51). On the basis of limited DNA sequence analysis, the genomes are estimated to have diverged by around 8 to 20% (1, 29). The two species mate efficiently, but their meiotic spores are inviable (28, 33, 49). Hunter et al. (33) demonstrated that the 1% of spores which are viable had undergone low frequencies of genetic exchange and exhibited a high degree of aneuploidy. In *pms1* and *msh2* mutant hybrids, exchange was increased, missegregation of the homeologous chromosomes was reduced, and spore viability was improved.

The current work extends these studies by exploiting yeast diploids in which a single *S. paradoxus* chromosome III is present in an otherwise *S. cerevisiae* genetic background. This system permits a more detailed examination of the effects of homeology without the high degree of spore inviability associated with the full *S. paradoxus-S. cerevisiae* hybrid diploid. We observe a reduction in genetic exchange between the homeologous chromosomes, accompanied by a reduction in spore viability. A fraction of this death is consistent with a meiosis I nondisjunction of the homeologous chromosomes. A further proportion of the inviability is a result of recombination-associated chromosome loss. The phenomena we describe are all dependent on an active mismatch repair system.

MATERIALS AND METHODS

Strains. The genotypes of the strains used are shown in Table 1. All strains are isogenic to either the *S. cerevisiae* Y55 (48) or the *S. paradoxus* N17 (50) parent. The Y55 markers *leu2-r, his4-r, thr4-a, trp1-b,* and *ura3-n* are restriction site fill-in mutations. *lys2-d* is a spontaneous mutation obtained by selection on α-aminoadipate medium (12). Other Y55 mutations were UV induced or spontaneous (48). The auxotrophic markers *ura3-1, lys2-1,* and *lys5-1* were introduced into N17, a wild-type isolate of *S. paradoxus*, by UV mutagenesis and subsequent selection on 5-fluoroorotic acid (5) and α-aminoadipate, respectively. These mutations were confirmed by complementation testing with the corresponding auxotrophic markers of Y55. Heterothallic versions of the wild-type N17 were obtained by creating a 100-bp *Pst*I deletion within the *HO* coding sequence. A *Hin*dIII fragment of the *S. cerevisiae HO* gene containing the deletion was cloned into a *URA3* selectable, integrative vector and introduced into a *ura3* N17 homothallic strain by two-step gene replacement (68). Yeast transformation was carried out by the lithium acetate method (21). Seven mating spores were isolated, and one was crossed to a homothallic N17 *lys2* strain; the spore viability of the resulting diploid was approximately 95%.

The left arm of chromosome III in Y55 was marked at *HML* with *ADE1*. The *Bam*HI fragment of pXW123 (80) was introduced into Y55-2291 by one-step gene transplacement (67), and transformants were confirmed by Southern analysis (71). The *kar1-Δ13* mutation was created by two-step gene replacement after linearizing pMR1591 (74) with *Bgl*II. Transformants were confirmed by the occurrence of an inefficient mating phenotype (13) and by Southern analysis. The *pms1Δ::URA3* mutation is a *URA3* replacement of the coding sequence of *PMS1* (42) and was introduced by one-step gene transplacement. The *msh2::URA3* mutation was created by transposon mutagenesis, and the *Spe*I fragment of pII-2-7 (61) was introduced by one-step gene replacement. The *pms1 msh2* double mutant was created by two-step gene replacement with the *Bst*XI fragment of pWK4Δ*pms1* (40) followed by one-step gene transplacement of the *Spe*I fragment of pII-2-7. All transformants were verified by occurrence of a mutator phenotype (61, 78) and by Southern blot analysis.

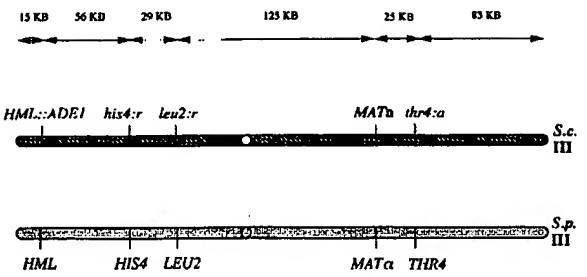

FIG. 1. Chromosome III genetic markers. Four intervals covering approximately two-thirds of the physical map of chromosome III from *S. cerevisiae* are monitored. The distances shown are the known physical distances between the markers of the *S. cerevisiae* chromosome.

Media and culture conditions. Strains were grown at 30°C on yeast extract-peptone-dextrose (YEPD) and synthetic complete media lacking one amino acid. Sporulation was induced on 2% potassium acetate–0.22% yeast extract–0.05% glucose–2.5% agar–0.09% complete amino acid mixture for 3 days at room temperature. Only asci with four spores were selected for tetrad dissection. Tetrad dissection and analysis were performed by standard techniques. Mismatch repair mutant strains were mated for approximately 6 h at 30°C on YEPD medium to minimize the accumulation of haplo-lethal mutations. This mating is 2 h longer than that described in the "zero growth" protocol of Kramer et al. (40). The longer mating was necessary to compensate for the poor mating efficiency of *kar1* strains.

Chromosome transfer by *kar1*. Strains that were partially hybrid for chromosome III were created by *kar1*-mediated single-chromosome transfer from N17 into Y55-2395 by a modification of previously described methods (18, 55). Chromosome transfer events were selected on synthetic medium lacking leucine and supplemented with cycloheximide (10 mg/liter). Strains disomic for chromosome III, which arise from the chromosome transfer event, were confirmed physically by the appearance of a band of double intensity by CHEF gel analysis (51) and genetically by a nonmating sporulation-deficient phenotype.

Analysis of disomy arising during meiosis. All tetrads with only three viable progeny arising from meiosis of the partial hybrid were screened by CHEF gel analysis for the presence of two copies (disomy) of chromosome III. Meiosis I nondisjunction events were determined by genetic analysis. These nondisjunction events yield tetrads with only two viable spores. Each of these spores contains one *S. cerevisiae* chromosome III and one *S. paradoxus* chromosome III and are therefore nonmating because of codominant expression of *MAT***a** and *MAT*α. Additionally, the presence of both chromosomes results in a wild-type chromosome III genotype because of complementation of the auxotrophic markers on the *S. cerevisiae* chromosome. A subset of this class of tetrads was confirmed to be disomic for chromosome III by CHEF gel analysis.

Selection for loss of the resident Y55 chromosome III. *S. cerevisiae* strains monosomic for the N17 chromosome III were constructed by transplacing the *Eco*RI fragment of pGEM7.10ΔCX*URA3* (a *URA3* disruption of the *MSH3* open reading frame, a gift from Giovanna Carignani) into the disomic strains obtained from chromosome transfer. Transplacement occurs preferentially into the Y55 copy of the *MSH3* gene because of reduced homology with the *S. paradoxus* chromosome. Subsequently, 5-fluoroorotic acid selection for *ura3*$^-$ strains was used to obtain haploids which had lost the resident Y55 chromosome. The resulting monosomic strains are α maters with the *S. paradoxus* chromosome III genotype.

Construction of a diploid homozygous for the *S. paradoxus* chromosome III. The mating type of the *S. paradoxus* chromosome III was switched by inducing the expression of the *HO* endonuclease on a vector under the control of a GAL promoter (34). Single colonies containing the plasmid were plated on media lacking uracil and containing galactose. The expression of *HO* then allows mating-type switching to occur. After a number of generations, colonies were shifted back to glucose medium and were cured of the plasmid. These strains were then scored for mating type. Only 1 of the 10 colonies screened had switched mating type, presumably because the *gal3* mutation in Y55 inhibits galactose uptake and therefore induction of *HO*. The switched strain was mated to SCt 14 to create SCD 39, a diploid in which both copies of chromosome III are from *S. paradoxus*.

Genetic and data analysis. Spore colonies from dissected asci of mismatch repair mutant strains were replica plated directly to synthetic media to observe the sectored colonies indicative of PMS events. Non-Mendelian segregations are gene conversions resulting in 6+:2− or 2+:6− segregation and PMS to produce 5+:3− or 3+:5− segregation.

Recombination in the partial hybrid diploids can be monitored in four genetic intervals, covering approximately two-thirds of the *S. cerevisiae* chromosome III physical map (Fig. 1). The genotype of the dead spore in those tetrads in which only three spores germinated can be predicted from the segregation of the genetic markers in the three surviving spores, assuming 2:2 segregation of all markers. In cases when an apparent crossover occurs between *HIS4-HML* and *MAT-THR4*, we cannot exclude the possibility that the recombinant genotype arose by a gene conversion of *HML* or *THR4*. However, we have included these potential gene conversions as crossovers when calculating map distances for two reasons. First, the overall frequency of gene conversion is very low, and therefore these events probably did arise from crossing over. Second, even if they did result from gene conversion, approximately half of all gene conversions are associated with a crossover (20).

The map distance in centimorgans is calculated as

$$\frac{(1/2)(\mathrm{TT} + 6\mathrm{NPD})}{\mathrm{PD} + \mathrm{TT} + \mathrm{NPD}}$$

where TT is tetratype, NPD is nonparental ditype, and PD is parental ditype.

Data sets were analyzed by the standard normal and G tests as described previously (70). Values of $P < 0.05$ were considered significant. Recombination frequencies among strains were compared by a G test on tetrad classes of 0, 1, 2, and more than 2 crossovers per tetrad.

In Results, we show that homeologous recombination is associated with the death of one spore in which a crossover had occurred. As a consequence, the relative frequency of crossovers in tetrads with three viable spores and four viable spores changes with respect to that found in the homologous wild-type control. We further show that in the absence of mismatch repair, this alteration in distribution of crossovers is nearly restored to the homologous wild-type pattern, and we thus conclude that the death observed in the wild-type partial hybrid is dependent on mismatch repair. However, the mismatch repair-defective strains (*pms1*, *msh2*, and *pms1 msh2*) have an increased frequency of asci with three viable spores (2, 40, 61, 78), and this death could obscure or enhance the genuine effect of mismatch repair mutations on the change in the distribution of crossovers. To determine whether the observed restoration is real or artifactual, we can estimate the number of tetrads with three viable spores which arose as a consequence of the mismatch repair mutations. We can also determine what proportion of the remainder are recombinant. Of those recombinants, we can then estimate the proportion whose death was associated with recombination. An example of this correction, using the data in Table 3 for the wild-type and *pms1* partial hybrids, is as follows. First, the proportion of tetrads with three viable spores with respect to the total tetrads with three and four viable spores must be calculated for the wild-type and *pms1* partial hybrids. For the wild-type partial hybrid, this value is 83/(83 + 357) or approximately 18.9%. For the *pms1* partial hybrid, the proportion of tetrads with three viable spores is 76/(76 + 140) or approximately 35.2%. This is an increase of approximately 16.3% (35.2% − 18.9%) compared with the wild type. Therefore, of the total asci in the *pms1* strain, 16%, or approximately 35.3 (0.16 × 216), are tetrads with three viable spores whose death was unrelated to homeologous recombination. Therefore, we subtract 35.3 from the observed 76 to leave 40.7 tetrads with three viable spores. If we assume that these tetrads with three viable spores have the same proportion of crossovers as found in the four-viable-spore class of tetrads, i.e., ~22% (31 of 140), then, of these 35.3, approximately 7.8 would have normal crossovers. These are removed from the 20 observed crossovers, leaving approximately 12.2 to be considered as having arisen from homeologous recombination. Therefore, the final frequency of crossing over in the three-viable-spore class of tetrads, excluding those which arose because of the increased death in a *pms1* background, is 12.2/40.7 [(20 − 8)/(76 − 35)] or 29.9%. Thus, the corrected ratio of crossing over in tetrads with three viable spores to crossing over in tetrads with four viable spores is 29.9%:22.1% or 1.35:1. A final correction is required to determine if crossovers in this class of tetrad are associated with death (as in the wild-type partial hybrid). In 12 of the 20 original crossovers, one of the two recombinant partners is in the dead spore. However, we have to remove the contribution of the 7.8 normal crossovers that we removed from the 20 original crossovers. Since we assume that the *pms1*-associated death is random, only half (3.9) of these 7.8 have a dead partner. Therefore, of the 12.2 (20 − 7.8) tetrads with three viable spores which are recombinant, 8.1 (12 − 3.9) or 66.4% have a dead recombinant partner. Similar manipulations can be applied to the other mismatch repair-defective strains.

RESULTS

***S. paradoxus* chromosome III can substitute for *S. cerevisiae* chromosome III.** A partial hybrid yeast was created as described in Materials and Methods. The observation that *S. cerevisiae* chromosome III can be eliminated from the disomic transfer strain indicates that the *S. paradoxus* chromosome possesses all of the essential chromosome III genes necessary for *S. cerevisiae* viability. The resulting haploid strain was subsequently mated to create the partial-hybrid diploid SCD 22. To determine if *S. paradoxus* chromosome III is fully functional in the *S. cerevisiae* genetic background during meiosis, a diploid in which both copies of the chromosome were derived from *S. paradoxus* (SCD 39) was constructed. The high spore

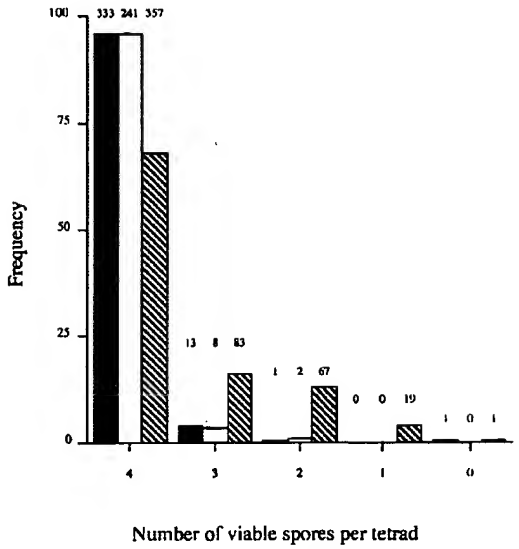

FIG. 2. Spore viability. The increase in the inviability of the partial hybrid (▨) relative to the homozygous *S. cerevisiae* control (■) is indicative of a nonrandom pattern of spore death. The diploid SCD 39 (□) contains two copies of chromosome III derived from *S. paradoxus* in an otherwise isogenic *S. cerevisiae* genetic background. This diploid exhibits normal, intraspecific levels of spore viability. Numbers above each bar represent the total numbers of asci of each class.

viability observed in this case (Fig. 2) indicates that the homologous *S. paradoxus* chromosome III pair is processed efficiently by the *S. cerevisiae* segregational apparatus. Indeed, the pattern of viability is not significantly different from that of the *S. cerevisiae* control. We would predict that normal frequencies of crossing over occur in this diploid. However, this could not be confirmed because of the absence of genetic markers on *S. paradoxus* chromosome III.

The fertility of the partial hybrid is reduced. The spore viability of SCD 22 is significantly reduced ($P < 0.001$) relative to the homozygous control *S. cerevisiae* diploids, SCD 24 and SCD 35. The spectrum of viability indicates a nonrandom pattern of death, $P < 0.001$ (Fig. 2). Of 527 tetrads dissected, 357 asci had four viable spores (67.7%), 83 had three (15.7%), 67 had two (12.7%), 19 had one (3.6%), and 1 had none (0.19%) (Fig. 1).

Meiotic crossing over is reduced between homeologous chromosomes. Meiotic recombination in the partial hybrid can be monitored in four genetic intervals, covering approximately 250 kb of the 320-kb chromosome III (Fig. 1). The frequency of exchange between the divergent chromosomes was determined by tetrad analysis. Recombination data were calculated from tetrads with four viable spores and from asci which yielded only three viable spores. For this latter class of tetrad, it is possible to predict the genotype of the dead spore from the segregation pattern of genetic markers observed in the remaining viable spores (see Materials and Methods). Data from these two classes of tetrad are presented in Table 2, and map distances for each interval are shown in Table 3. Recombination data for homologous mismatch repair-deficient strains were not determined, because previous control experiments have demonstrated that *pms1* and *msh2* mutants do not affect the rates of intergenic, meiotic recombination in a perfectly homologous diploid (33). Crossing over in the partial hybrid is suppressed in each of the four genetic intervals monitored. The *HML*-to-*HIS4* map distance is contracted 60-fold when compared with that of the control Y55 homozygous diploids (SCD 24 and SCD 35). A 47-fold reduction in exchange is observed in the *MAT-THR4* interval. Only one event is observed in the *HIS4-LEU2* region in the 440 tetrads analyzed. The smallest reduction is in the *LEU2-MAT* interval, which exhibits a nine-fold reduction in exchange. The average reduction over the entire interval from *HML* to *THR4* is 25-fold. The overall reduction is even more extreme, 40-fold, if we consider only the four-viable-spore tetrad class (Table 2).

Mismatch repair mutant partial hybrids are predicted to show elevated frequencies of recombination. A partial-hybrid diploid, homozygous for a deletion of the *pms1* gene, was constructed and tetrads were analyzed (SCD 23; Tables 2 and 3). Reciprocal exchange is increased in three of the four intervals monitored; over the combined *HML*-to-*THR4* region, there is a 2.5-fold increase over that in the wild-type partial hybrid. However, no stimulation of recombination is observed in the *MAT-THR4* region. Overall, the *HML*-to-*THR4* map distance remains more than 10-fold reduced relative to the homologous controls. An *msh2* partial hybrid was also constructed (SCD 30 [Tables 2 and 3]). Recombination in this strain is affected to a greater extent than in the *pms1* diploid. The map distance is expanded 5.5-fold in the *HML-THR4* interval relative to that in the wild-type partial hybrid. This represents a 4.5-fold reduction in recombination relative to the homologous controls. The frequency of exchange observed in the *msh2* mutant is significantly greater than in the *pms1* partial hybrid. Genetic exchange in a *pms1 msh2* double mutant (SCD 40 [Tables 2 and 3]) increases significantly compared with that in the *pms1* and *msh2* partial hybrids ($P < 0.001$ and $P < 0.01$, respectively) over the whole *HML-THR4* interval. The map distance increases by 7-fold in the double mutant relative to the wild-type partial hybrid and is only 3.5-fold reduced from the homologous control. The increase in map distance in the double mutant is the sum of the increases in the single mutants. The map distance of *HML-THR4* is 34.7 centimorgans (cM) in the double mutant compared with 11.8 and 26.6 cM in the *pms1* and *msh2* single mutants, respectively.

Homeology causes an increase in the number of tetrads with three viable spores which are not due to aberrant chromosome segregation. A significant proportion of the spore death in the wild-type partial hybrid is due to an increase in the number of tetrads which produce only three viable spores (see above). This class of tetrad cannot arise from a meiosis I nondisjunction, which results in tetrads with only two viable spores. Two other errors in chromosome segregation will lead to the inviability of just one spore. These are precocious separation of sister chromatids and meiosis II nondisjunction (64). Both of these phenomena would result in one of the three viable spores containing an extra copy of chromosome III. To eliminate these possibilities, all colonies arising from tetrads with only three viable spores from the wild-type partial hybrid were tested for the presence of two copies of chromosome III by CHEF gel analysis (see Materials and Methods). No chromosome III disomes were observed in 108 spores analyzed from 36 tetrads, excluding precocious separation of sister chromatids and meiosis II nondisjunction as causes of spore death in this class of tetrads.

Tetrads with three viable spores are enriched for recombination events. Genetic analysis of recombination events in the partial hybrid (SCD 22) reveals that recombinants are recovered approximately six times more frequently in the tetrads with three viable spores (Tables 2 and 4). This is in sharp contrast to wild-type homologous recombination, which occurs with equal frequency in asci with four and three viable spores (Table 2). Although some of these events may have arisen by

TABLE 2. Total meiotic recombination in tetrads with three and four viable spores

Strain	% Recombination in tetrad class[a]:								Total recombination %	
	HML-HIS4		*HIS4-LEU2*		*LEU2-MAT*		*MAT-THR4*			
	4 spores	3 spores	4 spores	3 spores	4 spores	3 spores	4 spores	3 spores	4 spores	3 spores
Wild-type homozygote	72.7 (242/333)	76.9 (9/13)	37.8 (126/333)	53.8 (7/13)	48.6 (162/333)	38.5 (5/13)	40.5 (135/333)	23.1 (3/13)	200[b] (665/333)	185[b] (24/13)
Wild-type partial hybrid	0.42 (3/357)	4.82 (4/83)	0.00 (0/357)	1.20 (1/83)	4.20 (15/357)	17.9 (15/83)	0.00 (0/357)	4.76 (4/83)	5.04 (18/357)	28.9 (24/83)
pms1 partial hybrid	5.71 (8/140)	9.21 (7/76)	3.57 (5/140)	5.26 (4/76)	12.9 (18/140)	9.21 (7/76)	0.00 (0/357)	1.32 (1/76)	22.1 (31/140)	26.3 (20/76)
msh2 partial hybrid	13.9 (19/137)	25.4 (15/59)	6.57 (9/137)	11.9 (7/59)	22.6 (31/137)	23.7 (14/57)	4.38 (6/137)	5.08 (3/59)	47.4 (65/137)	66.1 (39/59)
pms1 msh2 partial hybrid	18.4 (21/114)	24.4 (20/82)	12.3 (14/114)	18.3 (15/82)	29.8 (34/114)	31.7 (26/82)	2.63 (3/114)	3.65 (3/82)	63.2 (72/114)	78.0 (64/82)

[a] Recombination data are pooled from two independent diploids for each cross. Numbers in parentheses represent total numbers of reciprocal exchange events including twice the number of nonparental ditypes (nonparental ditypes were observed only in the wild-type homologous diploids SCD 24 + 35).

[b] Each tetrad in the wild-type homologous control had more than one crossover across the whole interval monitored, hence the >100% total recombination observed.

gene conversion, they are still indicative of a recombinational interaction and are therefore included in this analysis (see Materials and Methods). In 96% of the asci with three viable spores with a genetically observable recombination event, the interacting partner can be predicted to be in the dead spore (Table 4). This is significantly greater than the 50% expected if the death were random. These data suggest that the genetic interaction contributes to the death of one partner of the recombination event and hence to the excess of three viable spores. As discussed more thoroughly below, we propose that it is the attempted formation of heteroduplex DNA and not the resolution of the Holliday junction intermediate which leads to this outcome, and therefore both gene conversions with and without crossing over (Fig. 3B and C) can contribute to this death. The loss of one reciprocal recombinant is alleviated in *pms1*, *msh2*, and double-mutant backgrounds. Exchange in these strains is evenly distributed between tetrads with three and four viable spores. In approximately 50% of cases, both reciprocal recombinant partners are recovered in two of the three viable spores. The data are presented in Table 4 in two ways. The observed frequency of recombination in the two classes of tetrads is shown, as are the ratios of recombination between the two classes. In addition, the data have been corrected in the mismatch repair mutant partial hybrids to account for the background death of these strains (see Materials and Methods).

Nondisjunction of chromosome III homeologs. The expected class of tetrads arising from a meiosis I nondisjunction event is that containing asci with two viable spores. Therefore, spore colonies from this class were analyzed for missegregation of chromosome III. Consistent with the meiosis I nondisjunction of chromosome III, which carries the mating-type locus, these spore pairs were both nonmating and sporulation deficient and contained genetic markers from both parental chromosomes. In Table 5, frequencies of nondisjunction per meiosis are expressed as the number of asci meeting these criteria as a fraction of total tetrads dissected. The increase in reciprocal exchange associated with the *pms1* and *msh2* partial hybrids improves the segregation of the homeologous chromosomes during meiosis. We observed a decrease in the rates of nondisjunction of the homeologs relative to those in the partial hybrid (SCD 22) ($P < 0.01$). Although the frequency of tetrads with only two viable spores increases in the mismatch repair-deficient strains (data not shown), these do not contain chromosome III disomes and hence the rate of nondisjunction per meiosis decreases. Although nondisjunction rates for the mismatch repair mutant strains are not significantly different from each other, this probably reflects the size of the data set. However, the correlation between increased crossing over and reduced rates of nondisjunction is maintained.

Non-Mendelian segregation in wild-type and mismatch repair-defective partial hybrids. The analysis of non-Mendelian segregation in the partial hybrid is limited to asci producing four viable spores (Table 6). Non-Mendelian segregation is observed in all the partial hybrids at *his4*, *leu2*, and *thr4* on chromosome III, as well as at *HML*::*ADE1* and the *MAT* locus. Gene conversion frequencies in each of the partial hybrid diploids are significantly reduced from that in the homologous controls (Table 6), with the exception of *HML*::*ADE1*. This exception may be because the *ADE1* marker at *HML* can undergo ectopic recombination with the resident *ADE1* locus on chromosome I. Of the gene conversion events at *his4* in the wild-type partial hybrid, 62.5% are associated with a reciprocal exchange of the flanking markers *HML*::*ADE1* and *leu2*, while at *leu2* the association with crossing over of the flanking *his4* and *MAT* markers is 57%. These associations lie within the

TABLE 3. Genetic map distances

Strain	Map distance (cM) of genetic interval[a]:					Fold reduction
	HML-HIS4	*HIS4-LEU2*	*LEU2-MAT*	*MAT-THR4*	Total *HML-THR4*	
Wild-type homozygous control (SCD 24 + 35)	47.4 (251/346)	22.2 (133/346)	29.3 (167/346)	20.8 (138/346)	120	1.0
Wild-type partial hybrid (SCD 22 + 28)	0.80 (7/440)	0.11 (1/440)	3.41 (30/440)	0.46 (4/440)	4.8	25.0
pms1 partial hybrid (SCD 23 + 29)	3.47 (15/216)	2.08 (9/216)	5.79 (25/216)	0.46 (1/216)	11.8[b]	10.2
msh2 partial hybrid (SCD 30 + 37)	8.70 (34/196)	4.1 (16/196)	11.5 (45/196)	2.30 (9/196)	26.6[b]	4.51
pms1 msh2 partial hybrid (SCD 40)	10.46 (41/196)	7.4 (29/196)	15.3 (60/196)	1.53 (6/196)	34.7[b]	3.46

[a] Map distance in centimorgans (cM) is calculated as described in Materials and Methods.
[b] Values significantly deviating from those for the wild-type partial hybrid ($P < 0.001$).

range observed previously (20). The total frequency of non-Mendelian segregation in the mismatch repair mutant partial hybrids does not increase significantly relative to that in the wild type. However, this is probably a reflection of the small size of the data sets.

DISCUSSION

Suppression of genetic exchange increases nondisjunction of homeologous chromosomes. Spore viability is reduced from 98% in a intraspecific diploid to 85% in the partial hybrid. There is an increase in the number of tetrads which yield two or three viable spores. Genetic analysis of the tetrads with only two viable spores reveals that they arose by meiosis I nondisjunction. Previous studies with many different organisms have illustrated the dependence of segregation on crossing over (reviewed by Hawley [26]), and our analysis supports this relationship.

Meiotic recombination is limited to specific intervals on the homeologs. Crossing over is negligible in the *HIS4-LEU2* and *MAT-THR4* intervals, with the majority of events being observed between *MAT* and *LEU2*. We suggest that these differences may be attributable to differences in the degree of sequence divergence among these intervals. For example, it is known that the region between *HIS4* and *LEU2* is not essential (65) and may vary considerably even among *S. cerevisiae* isolates (77). Structural differences between the homeologs may explain some of the variation in recombination rates in the different genetic intervals. These may include the presence of large nonhomologies such as Ty elements, as well as undetected chromosomal rearrangements. Confirmation of these possibilities awaits detailed sequence analysis of *S. paradoxus* chromosome III.

Failure to recombine the homeologous chromosomes results in nondisjunction in approximately 9% of meioses. This is significantly greater than the rate of nondisjunction for chromosome III in a homozygous diploid (44) and correlates well with the previously observed rate of nondisjunction (9.7%) from an analysis of the full hybrid (33). The rates of nondisjunction we observe, however, appear to be much lower than one would expect from such a dramatic reduction in crossing over. One possible explanation for this discrepancy is that a functional crossover may have occurred in the third of the chromosome that was not monitored genetically. Alternatively, the efficient segregation of homeologs which have not undergone a genetically observable crossover is consistent with the existence of distributive pairing. This mechanism has been

TABLE 4. Distribution of recombination events in asci with three and four viable spores

Strain	% Recombination in tetrad class:		Ratio of recombination in three-viable class to recombination in four-viable class	% of three-viable class with exchange in which the dead spore was recombinant[a]
	Three viable spores	Four viable spores		
Wild-type homologous control (SCD 24 + 35)	185[b] (24/13)	200[b] (665/333)	0.93:1.00	37.5 (9/24)
Wild-type partial hybrid (SCD 22 + 28)	28.9 (24/83)	5.04 (18/357)	5.73:1.00	95.8 (23/24)
pms1 partial hybrid (SCD 23 + 29)	26.3 (20/76) 29.9[c]	22.1 (31/140)	1.19:1.00 1.35:1.00[c]	60.0** (12/20) 66.4[c]
msh2 partial hybrid (SCD 30 + 37)	66.1 (39/59) 77.3[c]	47.4 (65/137)	1.39:1.00 1.63:1.00[c]	48.7*** (19/39) 48.3[c]
pms1 msh2 partial hybrid (SCD 40)	78.0 (64/82) 96.2[c]	63.2 (72/114)	1.23:1.00 1.52:1.00[c]	65.6*** (42/64) 78[c]

[a] The frequency of death of one recombinant spore differs significantly in mismatch repair-deficient diploids relative to the wild type: **, $P < 0.01$; ***, $P << 0.001$.
[b] As in Table 2, each tetrad in the wild-type homologous control had more than one crossover across the whole interval monitored, hence the >100% total presented.
[c] Values corrected for the background death of the mismatch repair mutants (see Materials and Methods).

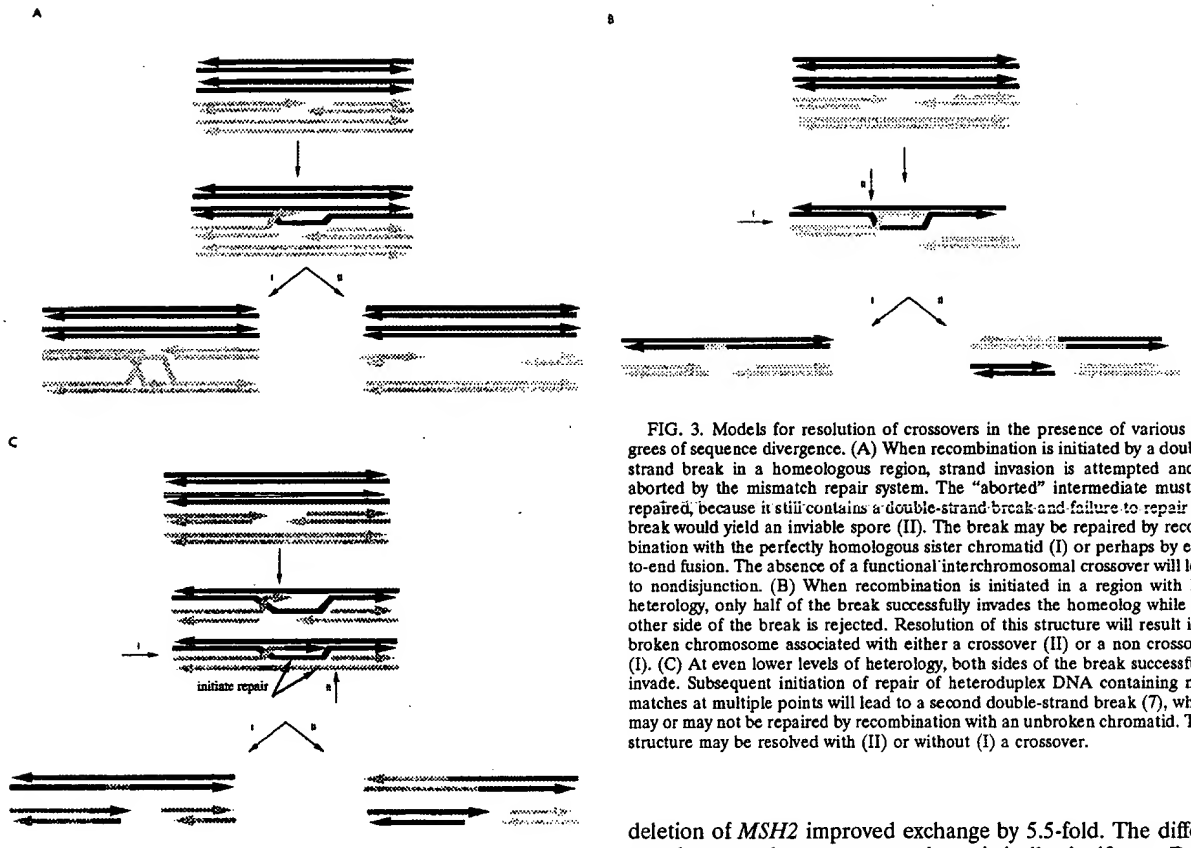

FIG. 3. Models for resolution of crossovers in the presence of various degrees of sequence divergence. (A) When recombination is initiated by a double-strand break in a homeologous region, strand invasion is attempted and is aborted by the mismatch repair system. The "aborted" intermediate must be repaired, because it still contains a double-strand break and failure to repair the break would yield an inviable spore (II). The break may be repaired by recombination with the perfectly homologous sister chromatid (I) or perhaps by end-to-end fusion. The absence of a functional interchromosomal crossover will lead to nondisjunction. (B) When recombination is initiated in a region with less heterology, only half of the break successfully invades the homeolog while the other side of the break is rejected. Resolution of this structure will result in a broken chromosome associated with either a crossover (II) or a non crossover (I). (C) At even lower levels of heterology, both sides of the break successfully invade. Subsequent initiation of repair of heteroduplex DNA containing mismatches at multiple points will lead to a second double-strand break (7), which may or may not be repaired by recombination with an unbroken chromatid. This structure may be resolved with (II) or without (I) a crossover.

observed in *D. melanogaster* (22, 27) and in *S. cerevisiae* (15, 23, 35, 43, 45). Without a chromosome marked genetically from telomere to telomere, however, we are unable to distinguish these two possibilities.

The mismatch repair genes *PMS1* and *MSH2* inhibit meiotic homeologous recombination to different extents. The observation that non-Mendelian segregations occur in the partial hybrids suggests that heteroduplex DNA is formed during exchange between the diverged sequences. We can conclude that homeologous recombination proceeds via a pathway involving heteroduplex formation. The data presented here and in other studies (14, 16, 19, 33, 60) indicate that the mismatch repair system plays a major role in preventing recombinational interactions between homeologous sequences. We observed that deletion of the *PMS1* gene increased meiotic crossing over between the homeologous chromosomes by 2.5-fold while a deletion of *MSH2* improved exchange by 5.5-fold. The difference between the two mutants is statistically significant. Data from a study of mitotic homeologous recombination (14) indicate that these two mismatch repair proteins have different effects at different levels of heterology. A *pms1* mutant strain in this study shows a 10-fold elevation in recombination rates at 91% homology while exhibiting only a 2-fold increase with a 77% homologous substrate. Recombination rates in the *msh2* mutant increased 70- and 6-fold respectively. The observed disparity between *PMS1* and *MSH2* during homeologous recombination is consistent with the recombination and segregation data obtained in the study of the *S. cerevisiae-S. paradoxus* hybrid (33). Additional data to be presented elsewhere (32) indicate that *PMS1* plays a lesser role in preventing exchange between diverged sequences during meiotic recombination.

To determine if *PMS1* and *MSH2* are operating in the same or different pathways during homeologous exchanges, a *pms1 msh2* double-mutant strain was constructed. Because Pms1p and Msh2p are proposed to act in concert (2), we would have

TABLE 5. Meiosis I nondisjunction of chromosome III in partial hybrids

Strain	No. of pairs of disomes[a]	Total no. of asci dissected	Frequency of chromosome III nondisjunction (%)[b]
Wild-type partial hybrid (SCD 22 + 28)	49	527	9.30
pms1 partial hybrid (SCD 23 + 29)	17	336	5.06**
msh2 partial hybrid (SCD 30 + 37)	11	255	4.31**
pms1 msh2 partial hybrid (SCD 40)	9	312	2.88***

[a] Disomes are assigned by a nonmating, sporulation-deficient phenotype (see Materials and Methods).
[b] The frequencies of nondisjunction of chromosome III in the mismatch repair mutant partial hybrids are significantly lower than in the wild-type partial hybrid: **, $P < 0.01$; ***, $P < 0.001$.

TABLE 6. Non-Mendelian segregation of genetic markers on chromosome III in the partial hybrid

Diploid	No. (%) of events at genetic marker[a]:									
	HML::ADE1		*his4-r1*		*leu2-r1*		*MAT*		*thr4-asp*	
	6:2/2:6	5:3/3:5	6:2/2:6	5:3/3:5	6:2/2:6	5:3/3:5	6:2/2:6	5:3/3:5	6:2/2:6	5:3/3:5
SCD 24 + 35 (wild type)	1/350 (0.29)	0/350 (0)	29/350 (8.29)	0/350 (0)	7/350 (2)	0/350 (0)	4/350 (1.14)	0/350 (0)	3/350 (0.86)	0/350 (0)
SCD 22 + 28 (partial hybrid)	5/357 (1.40)	0/357 (0)	1/357 (0.28)	0/357 (0)	2/357 (0.56)	0/357 (0)	0/357 (0)	0/357 (0)	1/357 (0.28)	0/357 (0)
SCD 23 + 29 (*pms1*)	2/140 (1.43)	0/140 (0)	1/140 (0.71)	0/140 (0)	0/140 (0)	0/140 (0)	1/140 (0.71)	0/140 (0)	0/140 (0)	0/140 (0)
SCD 30 (*msh2*)	2/137 (1.46)	0/137 (0)	1/137 (0.73)	1/137 (0.73)	2/137 (1.46)	0/137 (0)	1/137 (0.72)	0/137 (0)	1/137 (0.73)	1/137 (0.73)
SCD 40 (*pms1 msh2*)	1/114 (0.87)	0/114 (0)	0/114 (0)	1/114 (0.88)	3/114 (2.63)	1/114 (0.88)	1/114 (0.87)	0/114 (0)	3/114 (2.63)	0/114 (0)

[a] Values presented are total numbers of events at each marker as a fraction of the total number of asci with four viable spores. 6:2/2:6 indicates gene conversion events, and 5:3/3:5 indicates PMS events. The partial hybrid is statistically different from the wild-type homologous control diploid (SCD 35), $P << 0.001$.

predicted that a double mutant would be no more severe than either mutant alone. Analysis of post-meiotic segregation frequencies (2) and rates of mitotic mutation (46, 78) support this proposal. However, with respect to meiotic homeologous recombination, the phenotype of the double mutant is more severe. The total increase in homeologous recombination in the double mutant significantly exceeds the rates of exchange observed in either the *pms1* or *msh2* strain ($P < 0.001$ and $P < 0.01$, respectively). In fact, the rates of exchange in the double mutant are additive. This observation is consistent with the hypothesis that *PMS1* and *MSH2* can act independently to inhibit homeologous exchanges. Meiotic recombination studies with known levels of heterology (32) suggest that the density of mismatched base pairs dictates whether a *PMS1*-dependent or -independent pathway is employed to process the heteroduplex intermediate. Consistent with the disparate roles of *MSH2* and *PMS1* is the observation that murine *msh2* and *pms2* (the murine *PMS1* homolog) mutants (3, 16) have different meiotic phenotypes. Alternatively, Pms1p may enhance the inhibitory effects of Msh2p. This has been suggested by Worth et al. (79) from the observation that MutL enhances the inhibitory effect of MutS on RecA-catalyzed strand transfer in the presence of mismatches.

Exchange in the mismatch repair mutants is not restored to the rates observed for homologous sequences. There are a number of possible explanations for this observation. For example, RecA-catalyzed strand exchange is blocked by homeologous sequences in vitro (79). In support of a critical role of RecA in homeologous recombination is the observation that the overexpression of RecA during the SOS response (47) facilitates interspecies recombination in vivo. It is possible that the yeast RecA homolog(s) is limiting when exchange is attempted between the homeologous sequences. Another possibility is that the amount of initiation of meiotic crossing over is reduced between homeologous chromosomes. Heterologies have been observed to decrease the level of meiosis-specific double strand breaks at two loci in *S. cerevisiae* (63, 81). Preliminary data from our own studies (11) indicate that the pattern of breaks on the *S. cerevisiae* chromosome is not altered in the presence of the homeologous chromosome III, although no quantitative data have yet been obtained. A third possibility is that other mismatch repair proteins partially substitute for the lack of *PMS1* and *MSH2*. Although Prolla et al. (57) have shown that yeast *mutL* homologs *MLH1* and *PMS1* interact in vitro, suggesting that they form a heterodimer, it is possible that homodimers form and possess some activity in vivo. Finally, any structural blocks to recombination, such as Ty insertions, would presumably be unaffected by a deficiency in mismatch repair.

Spore inviability is also associated with mismatch-stimulated, recombination-dependent chromosome loss. The decrease in spore viability of the partial hybrid proceeds through two pathways. One leads to asci with two viable spores that are disomic for the homeologous chromosomes as discussed above. The other pathway culminates in the loss of just one spore. This class of tetrads does not arise from precocious separation of sister chromatids or meiosis II nondisjunction (see Results).

Although suppressed in the partial hybrid, recombination is significantly enriched in asci with three viable spores (Tables 3 and 4). Approximately 30% of these asci have a genetically detectable recombination event, whereas only 5% of tetrads with four viable spores are recombinant. If exchange is randomly distributed between the two classes of tetrads, we would observe equal frequencies of recombinants in these two classes of asci, as in the wild-type homologous strain. The observed distribution deviates significantly from this expectation ($P << 0.001$). Mutations in both *PMS1* and *MSH2* not only increase exchange but also remove the bias toward recombination in asci with three viable spores, indicating a dependence for this phenomenon on the activity of these proteins. The ratio of crossing over between the two classes of tetrads in the mismatch repair mutant partial hybrids is not different from 1:1 (Table 4) as in the wild-type homologous control diploid. This restoration of the homologous distribution of crossovers is not an artifact of the death associated with the mismatch repair mutants. When the data are corrected for this death, the effect is still apparent.

Nearly all (96%) asci with three viable spores that have a recombination event have only one recombinant spore. This suggests that the interacting partner is in the dead spore. This phenomenon is not limited to chromosome III homeologs, because it has also been noted by Casey in a study of chromosome X homeologs from *S. carlsbergensis* (10). We suggest that this is related to a failure to successfully complete reciprocal crossing over.

Role of the mismatch repair system in homeologous recombination. Mismatch repair has been implicated in both the destruction and modification of recombinational intermediates containing heterologies. Rayssiguier et al. (60) and Alani et al. (2) have proposed that mismatch repair proteins cause the unwinding or rejection of heteroduplex DNA containing mismatches, a process termed antirecombination or heteroduplex rejection. Doutriaux et al. (17) have shown that the mismatch repair system is responsible for the reduced infectivity of unmethylated phage lambda containing mispaired DNA. In *E. coli*, methylation provides the basis for strand discrimination of mismatch repair during replication. In its absence, repair is

initiated on both the template and nascent strands, leading to double-strand breaks (76) and loss of infectivity. It is proposed that the DNA molecule is "killed" by indiscriminate converging of excision repair tracts, a process termed mismatch-stimulated killing (17). On the basis of the observation that as little as 0.1% sequence divergence can alter the type of recombinant recovered, Borts and Haber (7) postulated that similar convergent repair tracts could occur during meiotic recombination in *S. cerevisiae*. Repair of the double-strand break by a second round of recombination would alter the outcome of the event. Support for this hypothesis comes from the observation that a mutation in *PMS1* restores the pattern of recombination to that found when all sequences are homologous (8).

Elements of the mechanisms described above can be envisaged to be occurring during homeologous recombination in the present study. We suggest that the probability of a successful strand invasion is a function of the degree of divergence which is assessed by the mismatch repair proteins when heteroduplex DNA is formed. Thus, with near-perfect homology, the probability of a strand invasion is unity; therefore, the probability of a successful recombination event, dependent on two invasions, is also unity. This event will be either a crossover or a noncrossover depending on the resolution of the Holliday junctions. As divergence increases, the probability of successful strand invasion decreases, to ultimately reach zero at some, as yet undefined point of sequence divergence (Fig. 3A). Between these two extremes, the probability of a single, successful strand invasion is greater than zero but less than unity. However, the probability of both strands successfully invading is the square of the probability of a single successful invasion. The resolution of the structure formed by a single invasion (Fig. 3B) in the crossover configuration leads to a single recombinant chromosome whose partner is broken and hence nonviable, as is observed in the tetrads with only three viable spores. Resolution of the Holliday junction in the other orientation leads to a potentially detectable gene conversion or PMS event and a noncrossover chromosome (Fig. 3B), as well as a broken chromosome. Thus, even those spores whose definition as recombinant is dependent on potential gene conversion of one of the distal markers (see Materials and Methods) can also lead to tetrads with three viable spores. This model is similar to "half-crossover" models that have been proposed to account for recombination in the *E. coli* RecG pathway (38, 39, 41, 72, 82, 83), in yeast *RAD52* independent mitotic recombination (24), and in *Ustilago recI* mutants (37). At the levels of heterology, where both strands can form a stable heteroduplex (Fig. 3C), mismatch-stimulated killing may occur. The processing of mismatches by the mismatch repair machinery may lead to convergent excision repair tracts and a double-strand break (7, 8). Failure to repair the lesion results in a broken chromosome and a dead spore.

The role that the mismatch repair system plays in recombination is clearly complex. How the mismatch repair system assesses the degree of homology has not yet been elucidated. Although the molecular mechanism of mismatch correction during prokaryotic DNA synthesis is well understood (19), the discovery of multiple homologs of the bacterial genes has made the equivalent understanding in eukaryotes more difficult. Furthermore, the relationship between the mechanism of replication error correction and the processing of mismatches arising during recombination is unclear. The possibility that different complexes of the various mismatch repair proteins function at different levels of heterology as well as for different types of mismatch (46) must be considered. The observation in this study that the *pms1 msh2* double mutant has a stronger phenotype than either mutant alone is suggestive of more than one process.

The work presented here supports and extends the observations of Hunter et al. (33), who proposed that the mismatch repair system plays a role in the evolution and speciation of eukaryotes. This study demonstrates that a novel pathway of death occurs when diverged sequences attempt to recombine and that this also contributes to the meiotic sterility of hybrids.

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